Lead and Copper in Pigeons (*Columbia livia*) Exposed to a Small Arms–Range Soil

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Abstract Small arms–range (SAR) soils can be contaminated with metals from spent copper (Cu)-jacketed bullets. Avian species are particularly at risk because they are exposed to lead (Pb) through ingestion of grit, soil intake from preening, or ingestion of contaminated food near ranges. Examination of the effects of Pb on birds at ranges have mainly focused on intake and toxicity of Pb shot pellets or fragments; however, Pb in soils may be an important pathway of exposure. To evaluate the uptake and effects of Pb from an actual range, the soil fraction (<250 μm) from a contaminated SAR soil was used to dose pigeons (*Columbia livia*) for 14 days at low (2700 μg Pb and 215 μg Cu/d) and high (5400 μg Pb and 430 μg Cu/d) doses. At the end of the study, blood Pb and erythrocyte protoporphyrin were determined, and tissues were analyzed for Pb and Cu. Results showed that Pb was absorbed in a dose–response manner in blood, tissues, and feathers, and erythrocyte protoporphyrin, a biomarker of early Pb effect, was increased at blood Pb levels >50 μg/dL. Four tissues showed differential retention of Pb, with kidney having the highest concentration followed by liver, brain, and heart, whereas Cu levels were not changed. To examine possible interactions with other metals, amendments of either Cu or tungstate were made to the soil sample. Although these amendments seemed to decrease the absorption of Pb, the results were ambiguous compared with sodium chloride controls. Overall, this study showed that intake of SAR soils contaminated with Pb and Cu causes an increase in Pb body burdens in birds and that the response can be modulated by amending soils with salts of metals.

There are approximately 3000 United States Department of Defense small-arms ranges (SARs) in the United States and an additional 9000 nonmilitary sites (United States Environmental Protection Agency 2005). These sites are typically contaminated with lead (Pb) and, to a lesser extent, copper (Cu) and are most often found at backstop or berm areas, where spent bullets oxidize and degrade into soil (Cao et al. 2003b). Liberated Pb from these bullets is sequestered by soil particles and is mostly confined to the top few inches of soil (Cao et al. 2003a), except where soil is transported by surface runoff or leaching to underlying aquifers occurs. This natural degradation and immobilization of Pb from bullets, although promoting containment, also results in high local concentrations in soils that underlie spent bullets. The combination of increased soil Pb with accompanying Pb fragments makes SAR soils

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potentially high-risk areas for resident and migrant wildlife (Interstate Technology Regulatory Council 2003).

Risk-management strategies for sites that contain SARs can vary depending on current and future use and whether the range is open or closed. For the most part, decisions are typically driven by human health risk pertaining to Pb (Interstate Technology Regulatory Council 2003). Options for Pb risk management for SARs include (1) leave the Pb in place, (2) remove the Pb from the soil, or (3) treat the Pb chemically to stabilize it. Leaving the Pb in place could occur when an area of high sensitivity would be damaged by the removal of soils; removing Pb from the soil could occur when the land use is changing (i.e., to residential use); and phosphate-based amendment could occur where SARs continue to be used, but containment is needed.

Compared with the single-receptor model for humans, ecologic risk assessments are complex, comprising multiple receptors and varieties of exposure scenarios. Thus, understanding exposure and absorption for each potential receptor is important. In addition, where institutional controls may ameliorate exposure for humans, they typically are not effective for wildlife, such as birds. For risk assessment, in addition to the hazard quotient, models that predict risk from ingestion of Pb particles as grit have also been developed (Peddicord and Lakind 2000). Whatever the model, improvements in estimates of absorption and distribution (under controlled exposure) can serve to significantly refine Pb exposure and risk estimates (Johnson et al. 2007).

Birds can be resident or migratory visitors at SARs and may breed in habitats in and around closed ranges. Thus, they provide relevant models for studies of Pb exposure, toxicology, and risk from range soils. Toxicology studies in birds have predominantly used soluble salts of Pb (Burger and Gochfeld 2000; Cory-Slechta et al. 1980; Ohi et al. 1980), and studies related to ranges have for the most part concentrated on Pb shot ingested by birds (Burger et al. 1997; Vyas et al. 2001; Vyas et al. 2000). There have been a few in situ–exposure studies (Johnson et al. 2007; Lewis et al. 2001; Vyas et al. 2000); however, none have examined metal absorption from contaminated SAR soils alone. Control and measurement of exposure with in situ studies is difficult; therefore, using real-world samples in a laboratory-based dosing experiment is a more convenient approach to the estimation of Pb uptake.

In the present study, soil samples taken from a closed SAR with high Pb contamination from spent bullets (Bannon et al. 2009) were used to orally dose pigeons daily for 14 days, after which measurements of Pb and Cu distribution, toxicity, and biomarkers of early biologic effect were taken. In a separate group, samples were additionally spiked with tungstate or Cu to examine the effect of metal changes on absorption. Tungstate has been proposed as a replacement for Pb-jacketed bullets and could potentially co-contaminate (with Pb) ranges, and Cu is an essential metal as well as a contaminant at ranges and could interact with intestinal uptake of Pb. The objectives of the study were (1) to assess the absorption and distribution of Pb and Cu after dosing with a real-world soil sample from an SAR and (2) to examine the effects of adding either tungstate or Cu on the absorption of Pb.

Methods

Study Design

A representative SAR soil from a previous study was used; the study soil had been sampled from a closed SAR in Maryland with high Pb levels in the surface soil (Bannon et al. 2009). The soil had been sieved to <250 μm and assayed for total metals and soil properties as follows: sandy loam in texture (pH 6.1), cation exchange capacity (CEC) 1.1 meq/100 g, and total organic carbon 1.9%. Metal content (as determined by inductively coupled plasma mass spectrometry [ICP-MS]) was 8389 ± 124, 18,013 ± 1329, and 1433 ± 33 μg/g for Fe, Pb, and Cu, respectively. Other metals, such as zinc (Zn), manganese, antimony, nickel (Ni), and zirconium, were present at 100-fold lower concentrations and were not expected to affect the absorption of Pb and Cu (Bannon et al. 2009). Before subsampling (to make doses), the soil was homogenized by rolling and thorough mixing. An automatic capsule filler (ProfillVet; Torpac, NJ) was used according to the manufacturer's instructions to fill gel capsules to capacity to make the high-dose 300-mg soil treatments (mean 298 ± 5 mg), which contained approximately 5403 μg Pb and 430 μg Cu. The 150-mg low-dose soil (mean 149 ± 1 mg) capsules were filled manually and contained 2700 μg Pb and 215 μg Cu (see Table 1). To make the Cu- or tungstate-amended soil, either Cu chloride or sodium tungstate were added to 100 g soil to yield final soil concentrations of 500 μg Cu/capsule or 1000 μg WO_4/capsule, respectively. Capsules were then filled from these mixtures. For the Pb acetate (PbAc) stock solution, 9.150 g $(CH_3COOH)_2Pb \cdot H_2O$ were dissolved in deionized (DI) water to yield a Pb stock solution giving 5000 μg Pb in 100 μL (PbAc high dose) or 2500 μg Pb (PbAc low dose) in 50 μL water. As a positive control for tungsten acetate and Cu chloride amendments, a NaCl group was included for the high-dose soil treatment only; NaCl was added to 100 g soil to yield approximately 1600 μg NaCl/capsule. Control birds received 100 μL DI water.

Animals

Male adult pigeons (*Columbia livia*) were obtained from an authorized vendor and group housed until delivery. Birds

Table 1 Study design: All doses administered in a gel capsule

Treatment	Description
Control	100 µL DI water
Soil low dose	150 mg SAR soil containing Pb (2700 µg) and Cu (215 µg)
Soil high dose	300 mg SAR soil containing Pb (5400 µg) and Cu (430 µg)
Soil low dose +Cu	150 mg soil (soil low dose) with 250 µg Cu^{2+} added as $CuCl_2 2H_2O$
Soil high dose +Cu	300 mg soil (soil high dose) with 500 µg Cu^{2+} added as $CuCl_2 2H_2O$
Soil low dose +W	150 mg soil (soil low dose) with 500 µg WO_4^{2-} added as Na_2WO_4
Soil low dose +W	300 mg soil (soil high dose) with 1000 µg WO_4^{2-} added as Na_2WO_4
Soil high dose +NaCl	300 mg soil (soil high dose) with 1600 µg of NaCl
PbAc low dose	50 µL solution containing 2500 µg Pb as $(CH3COO)_2Pb \cdot H_2O$ in DI water
PbAc high dose	100 µL solution containing 5000 µg Pb as $(CH3COO)_2Pb \cdot H_2O$ in DI water

were subsequently single housed but had proximity and visual contact with the other birds. They received food and water ad libitum and were exposed to a 12-hour light-to-dark photoperiod for the duration of the study. Weights were taken approximately every 5 days. Birds were randomized to treatment and divided into dose groups of five birds each, except for the control group ($n = 4$; see Table 1 for dose descriptions). All doses, either soil or water, were administered in a gel capsule, which quickly dissolves on contact with internal body fluids. Doses of 5400 or 2700 µg Pb in the soil-dose groups were approximately equivalent to body weight doses of 11.9 ± 0.79 and 5.8 ± 0.60 mg/kg/d, respectively. Birds were dosed by placing a capsule in the back of the mouth and closing the bill momentarily, thereby ensuring that the capsule entered the crop. Animals were monitored daily for any visible Pb toxicity (during feeding or preening and/or in feces). After 14 days of exposure, animals were killed on day 15 with carbon dioxide, after which cardiac blood sampling was performed. Blood was removed into ethylene diamine tetraacetic acid (EDTA)-anticoagulated vials for Pb and erythrocyte protoporphyrin (EP) analysis as well as routine hematology and serum clinical chemistry. For each bird, three tail feathers were sampled for Pb analysis. The outer left remiges was removed before dosing (F1), whereas at the end of the study the next proximate (F2) and the growing replacement for F1 (F3) were removed. At necropsy, organs were examined for gross pathology and stored on dry ice initially and then stored at $-80°C$ until metal analysis. The protocol was approved by the Public Health Command Institutional Animal Care and Use Committee before the start of the study. Investigators and technicians adhered to the following guidelines: Public Health Service Policy on Humane Care and Use of Laboratory Animals; United States Government Principles for the Use and Care of Vertebrate Animals Used in Testing, Research, and Training; and the Animal Welfare Act (http://www.nap.edu/readingroom/books/labrats/).

Clinical Chemistry and Hematology

Hemoglobin was determined from whole blood using a HemoCue hemoglobin photometer (HemoCue AB; Angelholm, Sweden). Whole-blood evaluation for red and white blood cells, packed cell volume, hematocrit, and erythrocyte volume was performed using instrumental and manual methods described by Gogal et al. (2002). Whole blood was centrifuged to obtain plasma for clinical chemistry. A VetTest chemistry analyzer (IDEXX Laboratories, Westbrook, ME) was then used to measure albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, calcium, creatine-kinase, glucose, lactate dehydroganase, phosphate, total protein, triglycerides, globulin, sodium, potassium, and chloride.

Analytic Chemistry for Blood Samples

EDTA-anticoagulated whole-blood samples were analyzed for Pb using a Perkin Elmer 4100ZL graphite furnace atomic absorption spectrometer based on a previously published method (Parsons and Slavin 1993). Briefly, samples were prediluted $(1 + 9)$ to 50 µL with modifier solution containing 0.2% w/v $NH_4H_2PO_4$, 0.5% v/v Triton X-100, and 0.2% HNO_3. The modifier allows for a high pyrolysis temperature and stabilizes Pb during atomization. Pb nitrate was used to make aqueous calibration standards with the same modifier. Sample aliquots, 12 µL each, were auto injected onto a L'Vov platform in a graphite tube. Quality-control materials were used throughout the procedure using fixed target values assigned by the New York State Blood Lead Proficiency Program for human samples. Erythrocyte protoporphyrin determination was based on a previously published method (Chisolm and Brown 1975; Parsons 1999). Pb inhibits incorporation of iron into the heme molecule by the enzyme ferrochelatase, causing increased levels of circulating Zn protoporphyrin. Briefly, heme compounds and porphyrins from whole-blood

samples were extracted into an ethyl acetate–acetic acid mixture followed by back extraction of porphyrins into hydrochloric acid solution, after which spectrofluorometric measurement of free erythrocyte protoporphyrin was performed. Standard clinical quality-control procedures were used throughout analysis.

Analytic Chemistry for Tissue Samples

Frozen pigeon tissues were thawed and dried in a vacuum oven at approximately 55°C overnight. Dry tissue samples, 0.05–0.1 g each, were placed into the microwave vessel for digestion. Then 1.0 mL concentrated hydrogen peroxide (30% Optima grade; Fisher Scientific, Pittsburg, PA), 1 mL deionized water (>18 MΩ cm; Millipore), and 1 mL concentrated nitric acid (70% Optima grade (Fisher Scientific) were added to each vessel. In addition, 0.1 mL 240-ppm gallium (Ga) was added before digestion as internal standard. All digested tissue samples were prepared in duplicate. Two digestion blanks and two control samples per batch were also prepared and microwave digested before analysis. Bovine liver (approximately 0.25 g) from the National Institute of Standards and Technology was used as standard certified material. Samples were reconstituted into acid-washed 15-mL polypropylene conical tubes to 12 mL with deionized water. Final digested samples had an internal standard of 2 ppm Ga. Digested samples were directly analyzed for Cu employing inductively coupled plasma–optical emission spectrometry (OES; Optima 3000 ICP-OES; Perkin Elmer) with a wavelength of 324.7 nm and further diluted 10-fold before they were analyzed for Pb using ICP-MS with a dynamic collision cell for the removal of polyatomic interferences (DRC II ICP-MS; Perkin Elmer). The calibration curve for Pb analysis consisted of 0-, 0.1-, 1.0-, 5.0-, and 20.0-ppb standards, and for Cu analysis it consisted of 0-, 0.05-, 0.2-, 1.0-, and 2.5-ppm standards. The limit of detection was 0.05 ppb for Pb and 0.007 ppm for Cu (based on measuring 20 blank solutions as samples), and analysis was monitored using quality-control samples.

Analytic Chemistry for Feather Samples

Feather samples were cleaned with DI water to remove external contamination, dried, and weighed. Ten milliliters of concentrated nitric acid (Optima grade; Fisher Scientific) was added to each sample, and digestions were performed using a CEM MDS-2100 microwave digestion system. After digestion, the samples were allowed to cool and then diluted to 50 mL with DI water. Samples were analyzed using a Hewlett Packard 4500 ICP-mass spectrometer. Stock solutions containing the elements of interest were purchased from Spex Certiprep (Metuchen, NJ). The instrument was calibrated using standards prepared in 20% nitric acid at concentrations that bracketed the expected concentration range of the samples. Internal standards were added using a mixing tee before the introduction of samples into the plasma. Continuing check standards and blanks were run at 10% frequency. Detection limits were set at 0.1 µg/L for the elements tested.

Other Sources of Pb and Cu

Other sources of Pb and Cu intake were accounted for in this study. Food contained 0.181 ppm Pb and 24.0 ppm Cu. Daily intake of Pb and Cu from food was estimated at 18 and 2400 µg, respectively (at an estimated 100 g food/d), and daily intake from dosing was approximately 5400 µg Pb and 430 µg Cu for the high-dose soil treatment, with an additional 500 µg Cu for the Cu-amended soil treatment (Cu+ or 1000 µg WO$_4$ for tungstate-amended soil [+W]). Pb intake from drinking water was <15 µg/L, which is the USEPA action level for human drinking water (http://www.epa.gov/safewater/contaminants/index.html#inorganic) (United States Environmental Protection Agency 2009).

Data Analysis

Figures were made using Prism® Software Version 4.02 (GraphPad, La Jolla, CA). Before analysis of variance (ANOVA) modeling, Shapiro–Wilk W test was used to verify normal distributions for treatment groups, and Welch's t-test showed that variances were equivalent between groups (JMP® version 8.0.2; SAS, Cary, NC). Two-way ANOVA was used to compare the two soil doses and the four treatment groups (i.e., control, CuCl$_2$, WO$_4$, and PbAc; Table 2) with respect to Pb and Cu in brain, heart, kidney, and liver tissues. This was followed by Bonferroni test to compare the treatment groups if the main effect was significant. Statistical significance was defined as $p < 0.05$ for all tests.

Results

Pigeons

There were no mortalities or moribund animals during the study. Figure 1 shows box plots of bird weights during the course of the study. Individual bird weights fluctuated (±5%) during the course of the study, but individual animals did not lose weight due to treatment or disease during the 14-day study. During the course of 14 days, a few birds had green feces, possibly indicating incipient poisoning, but overall there were no visible signs of toxicity (daily observations) in any of the treatment groups, and preening,

Table 2 Results of tissue analysis for Pb after nitric acid digestion and ICP-MS analysis

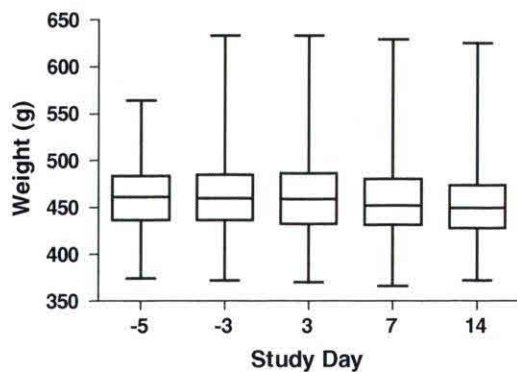| Dose group	Liver		Kidney		Heart		Brain	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
150 mg soil (2700 Pb/215 Cu)	9.91	2.11	22.74	3.17	0.36	0.08	1.67	0.47
300 mg soil (5400 Pb/430Cu)	11.75	1.04	47.76	9.77	0.44	0.03	2.32	0.3
150 mg soil + 250 µg Cu	4.18	0.56	14.5	2.11	0.18	0.02	0.84	0.12
300 mg soil + 500 µg Cu	16.21	2.35	33.58	5.46	0.46	0.11	2.7	0.48
150 mg soil + 500 µg W	7.06	0.83	18.19	2.81	0.26	0.08	1.15	0.25
300 mg soil + 1000 µg W	9.96	2.13	35.29	11.14	0.44	0.15	1.83	0.17
PbAc 2500 µg	7.99	0.87	23.79	4.23	0.37	0.04	1.4	0.15
PbAc 5000 µg	14.48	4.86	33.3	6.19	0.3	0.05	1.98	0.21
300 mg soil + 1600 µg NaCl	9.24	1.23	25.41	3.62	0.27	0.09	1.58	0.26

Results in µg/g dry-weight tissue. $N = 5$. There was a significant difference between all doses when high dose (300 mg soil) was compared with low dose (150 mg soil) treatment for each tissue. There were no treatment-related differences. $N = 5$ for all doses. The NaCl-amended group received only one dose and was not used in the two-way ANOVA

feeding, and general behavior were normal. The study design and dose group nomenclature are detailed in Table 1.

Analysis of Blood Samples

Figure 2a shows blood Pb concentrations (for all doses and treatment groups), which were analyzed using two-way ANOVA (two doses and four treatment groups). Control birds had blood Pb levels lower than the limit of detection, whereas birds given either 300 or 150 mg soil (unamended soil; soil) had a 2-fold difference in blood Pb values (89.0 ± 4.22 and 43.0 ± 4.28 µg/dL, respectively). This dose–response effect was also evident with Cu-amended soil (+Cu group). The addition of sodium tungstate to the soil (+W group) decreased PB absorption of birds receiving the high-dose treatment. Amendments using NaCl (1600 µg/capsule) also decreased Pb absorption in birds receiving the high-dose treatment. Although birds given similar amounts of soluble Pb (as PbAc) had similar blood
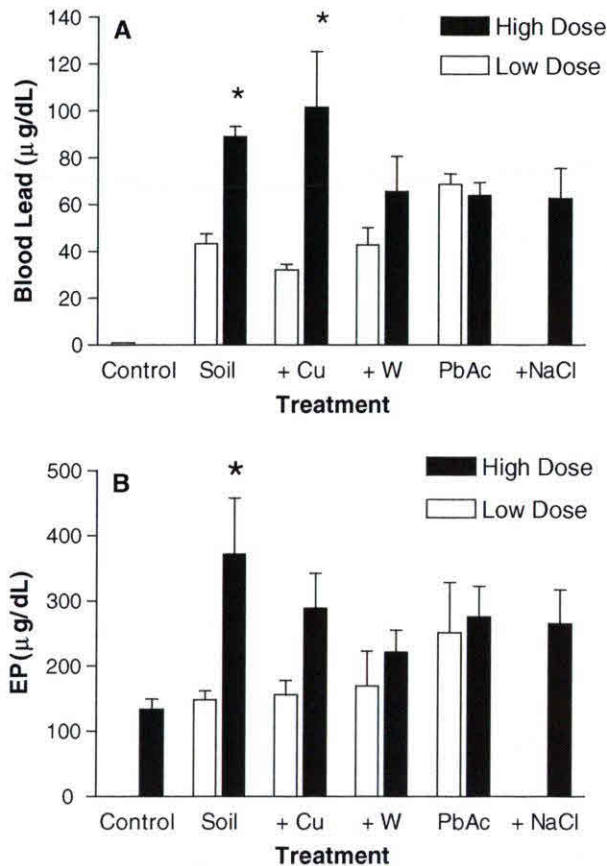
Pb values, this group did not demonstrate the dose–response effect evident in birds receiving soil alone. Statistically different blood erythrocyte protoporphyrin levels (shown in Fig. 2b) existed only in the soil-treatment group, whereas the addition of Cu or WO₄ decreased this effect. Again, as in the blood Pb treatments, there was no dose–response effect for EP regarding PbAc. Figure 3 shows the relation between blood Pb and EP for all samples in the study. Below approximately 50–60 µg/dL blood Pb, there was no effect on EP, but there was a clear dose–response effect at higher blood Pb values (approximately 50 µg/dL blood Pb), with increased variation between individuals at higher dose levels. Regarding clinical parameters, there was no difference between treatment groups for hematology (hematocrit, hemoglobin, erythrocyte, and leucocyte counts) or for serum clinical chemistry parameters: albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, calcium, creatine-kinase, glucose, lactate dehydroganase, phosphate, total protein, triglycerides, globulin, sodium, potassium, and chloride (data not shown).

Pb and Cu in Tissue Samples

Pb concentrations in tissues are listed in Table 2. Two-way ANOVA with dose (150 or 300 mg soil) and treatment (soil, +Cu, +W, PbAc) as factors was used to analyze the data. The NaCl group was not used in this analysis because there was no companion low-dose group. All tissues had statistically significant dose–response differences between the high- and low-dose treatments, with the overall highest differences observed in kidney, in which mean concentrations were 47 ± 9.77 and 22 ± 3.17 µg/g Pb for the two doses, respectively. Pb concentrations in organs (from highest to lowest) were as follows: kidney >liver >brain >heart. However, there was no statistically significant

Fig. 1 *Box plot* of weights of birds (in grams) during the course of the study ($n = 49$). Dosing began on day 0 (between day −3 and day 3)

Fig. 2 Blood Pb concentrations **a** and erythrocyte protoporphyrin **b** from the same birds after 14-day exposure to SAR soil contaminated with Pb. Bars are mean ± SE. * Statistically different from low-dose treatment at $p < 0.05$. Soil = SAR soil only. +Cu or +W = soil amended with either Cu or tungstate salts. +NaCl = 1600 µg sedum chloride added to high-dose treatment. PbAc = PbAc equivalent to soil concentration. The normal EP value measured for control birds was 134 ± 31 µg/dL, which was not different from that in the low-dose treatment

treatment effect. The combined results for both Cu and Pb (from Table 2) are shown in Fig. 4. Cu remained the same through all treatment groups. Mean Cu concentrations in control birds were 13.8 ± 1.41 (liver), 14.2 ± 0.82 (kidney), 19.90 ± 0.360 (heart), and 12.7 ± 2.13 µg/g (brain), and there was no difference between treatment groups.

Pb and Cu in Feathers

Feather metal analysis showed a clear dose–response effect for low- versus high-dose treatments regarding tail feathers that regrew (F3) during the 14-day study (Fig. 5a), whereas the feathers removed after the study (F2) had much lower concentrations. Metal concentrations in feathers from control birds (control group) were lower than the detection limit. Tail feathers from birds dosed with treated soil (1000 µg tungstate) showed decreased Pb levels (Fig. 5b)
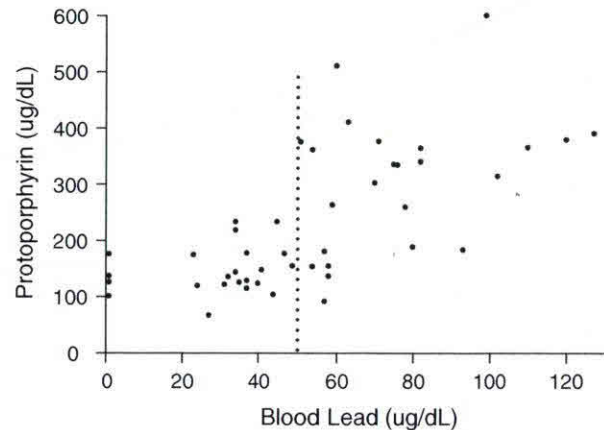
Fig. 3 Effect of lead ingestion on erythrocyte protoporphyrin in pigeons. Birds were exposed once daily to Pb in SAR soil for 14 days. Measurement of EP was by spectrofluorometry and blood Pb by graphite furnace atomic absorption spectrometry. Vertical line indicates arbitrary break point for linear response

compared with birds receiving soil-only treatment. Pb values in the high-dose treatment, with and without added tungstate, were 6.9 ± 3.9 and 4.1 ± 1.7 µg/g, respectively. There were no differences in Cu measurements in these feathers (data not shown). One feather (low Pb [F2]) was removed from analysis as an outlier because it had Zn and Cu levels that were 10-fold higher, as well as a higher Pb concentration, than feathers from the other birds.

Discussion

Birds are potentially frequent visitors to even inaccessible ranges and can accumulate metals by way of ingestion of soils while feeding, ingesting grit, or preening feathers. The most frequent concern for birds is Pb because it is both ubiquitous and persistent in soils, a legacy of past use of paint, gasoline, and munitions. On ranges, birds can be adversely affected by Pb poisoning after inadvertently ingesting Pb shot as grit (DeMent et al. 1987a; Hui 2004; Symes et al. 2004), an interesting parallel to Pb poisoning found in children, which is a factor in pica disorder (i.e., ingestion of nonfood objects) (Biddle 1982; Yaffe et al. 1984). A number of studies have assessed the toxicity of Pb in birds due to Pb shot ingestion (Carrington and Mirarchi 1989), whereas others have examined biomarkers of exposure (Johnson et al. 2007). However, few have examined uptake from SAR soils in a controlled-exposure experiment. This 14-day study examined the absorption of Pb and Cu by *C. livia* from daily doses of the <250 µm fraction of a representative SAR soil. The soil contained Pb and Cu from degraded bullets, with measured concentrations of 18,000 and 1400 µg/g, respectively. These high

Fig. 4 Tissue concentrations of Cu and Pb in pigeons after dosing with SAR soil. Bars represent mean ± SE for all tissues. Analysis was by acid digestion/ICP-MS. Values are expressed in µg/g dry weight. *Clear bars* = Cu measurements. *Hatched bars* = Pb measurements. Tissue is *color coded* and *bars stacked* for each treatment
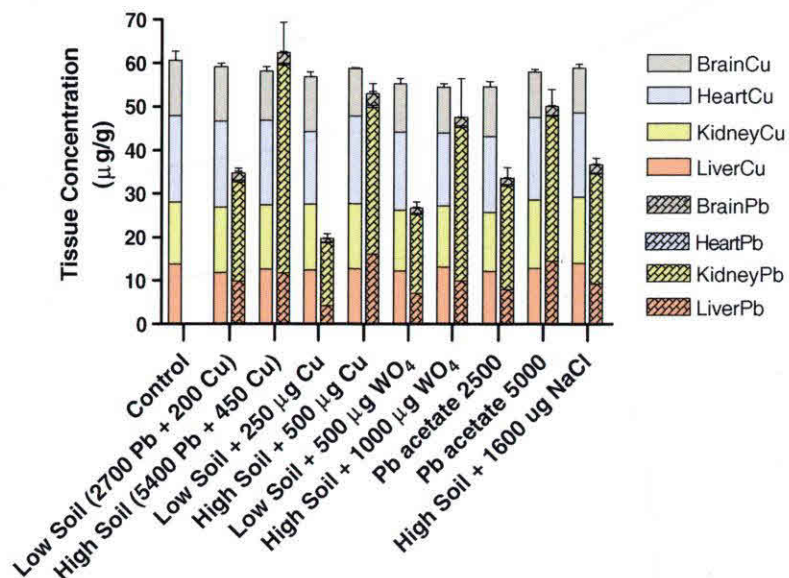
values resulted from both sampling hotspots on ranges as well as sieving. Other metals (Ni, Zn) were present in the soil, but at much lower levels, and were not expected to impact the levels of Pb and Cu absorbed by the birds. It should be noted that tissue metal concentrations were calculated on a dry-weight, which makes it difficult to directly compare our data with those of the majority of published studies, which measured tissue metals on a wet-weight basis.

Although no acute toxicity or changes in cell hematology or clinical chemistry were evident during this study, increased blood Pb levels were observed when birds were given either a low or high dose of the SAR soil alone (Fig. 2a, soil group). This was accompanied by increased EP in the high-dose group (2B, soil group). Both observations showed similar responses. Mean blood Pb value for the high-dose treatment was 89 ± 8.5 µg/dL Pb, which is comparable with 126 ± 14 µg/dL Pb found in Northern bobwhite fed with contaminated sediment (4500 µg/g in food) for 21 days (Connor et al. 1994). Although hemoglobin and hematocrit values remained normal (data not shown) at 14 days, the increased EP and blood Pb values found in this study indicate incipient Pb poisoning (Pain 1996). The EP value in control birds for this study was 134 ± 31 µg/dL, and the increased levels (371 ± 86 µg/dL, Fig. 2b) compare well with those measured in dark-eyed juncos at a skeet range (421 ± 40 µg/dL) (Vyas et al. 2000), an indication that the chosen doses and time frames were relevant. Interestingly, the response of EP to Pb had a threshold of approximately 50 µg/dL blood Pb (Fig. 3), showing that increased EP value is not a sensitive indicator of low-dose Pb exposure in pigeons. Blood Pb levels in live feral pigeons can range from 10.5 to 1870 µg/dL (DeMent

et al. 1987b), levels that are more often than not caused by Pb shot in the gizzard. Although it has been noted that pigeons seem to be more resilient to Pb poisoning than other birds, birds of prey can feed on Pb-poisoned animals (Miller et al. 1998), thereby increasing Pb body burdens in an interdependent food chain (DeMent et al. 1986).

The dose–response effect in blood Pb response between the low- and high-dose treatments was further reflected in the four tissues examined, with kidney having the highest concentrations, followed by liver, heart, and brain. Using two-way ANOVA with dose and treatment as factors, there was a significant dose-related (low vs. high) effect for all tissues, but treatment type (+Cu, +W) was not significant. The highest level in kidney (47.76 ± 9.77 µg/g) was similar to that in finches that had been dosed for 30 days with 25 ppm PbAc in drinking water (33 ± 13 µg/g Pb) (Dauwe et al. 2002). Certainly, kidney accumulates Pb more than other tissues and is affected by increasing Pb burdens (Beyer et al. 1988). However, for this study, clinical chemistry measures in serum did not differ between doses or treatment groups, perhaps because the study was of short duration or because the pigeons were resilient to increased Pb levels (Franson 1996).

It should be noted that the statistically significant differences between the high- and low-dose treatments found in the blood of pigeons exposed to unamended soil was eliminated with the addition of Cu or WO_4 (Fig. 2a), indicating a possible interaction between Pb and WO_4 or Cu with respect to absorption and/or tissue retention. Although WO_4 levels in tissues were not measured in this study, this interaction demonstrates that anions such as WO_4 could affect Pb absorption. Whether this is caused by decreased solubility in the gastrointestinal tract was not
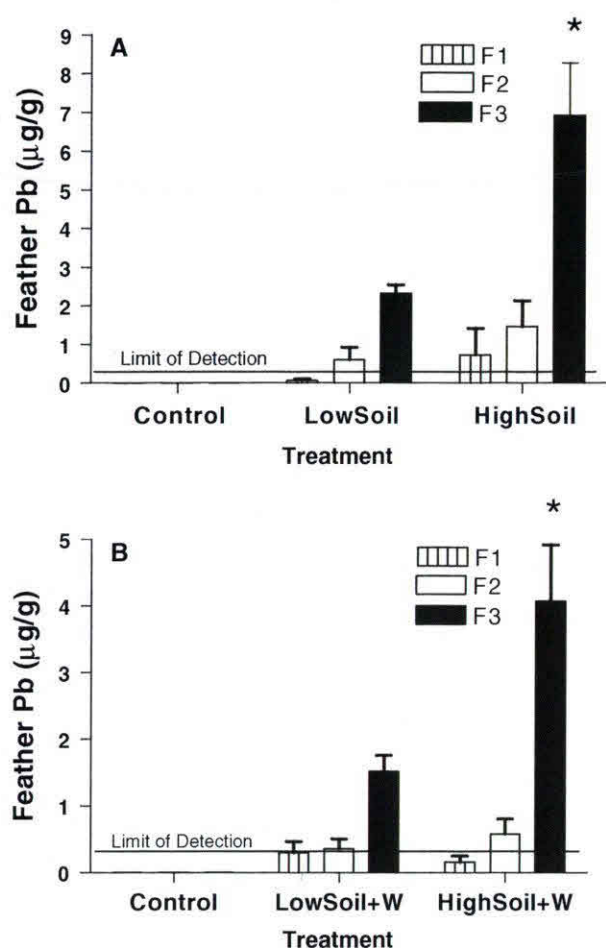
Fig. 5 Pb concentrations in feathers from pigeons after exposure to SAR soil contaminated with Pb. *Bars* are mean ± SE. F1 = tail feather pulled at start of study. F2 feather proximal to the regrown feather. F3 = regrown feather. **a** Feathers from birds dosed with SAR soil only. **b** Feathers from birds dosed with SAR soil treated with 1000 μg tungstate. The limit of detection was calculated based on average feather weight, dilution volume, and instrument detection limit

addressed by this study. The mechanism by which Pb (Bannon et al. 2003) or WO_4 (Cardin and Mason 1976) enter duodenal enterocytes and hence the body is not known, but it is expected to involve opportunistic use of existing nutrient transport mechanisms, and interaction could take place between metals at this site. Because there are no known ranges that are polluted with both Pb and W, co-contamination treatment was not available for this study. Finally, measured Cu levels were not different between controls, doses, or treatment groups (Fig. 4) using a Cu dose that was approximately one-third of the daily intake from food. This is not surprising because Cu has well-known molecular homeostatic mechanisms whereby excess intake can be quickly eliminated in bile by way of hepatic trafficking proteins (Barceloux 1999).

Feathers have been previously used to monitor birds for Pb exposure (Scheifler et al. 2006), and although qualitative results seem to be best with regrown feathers (Dauwe et al. 2002) (Burger et al. 1992), efforts to show dose–response effects to different exposures have been rare (Dauwe et al. 2002). Although other studies have investigated feather Pb concentrations in a risk-assessment context (Johnson et al. 2007), this work showed that Pb levels in newly grown feathers at two well-defined doses demonstrated a dose–response effect similar to Pb concentrations in blood and other tissues. Although blood Pb values are useful as monitors of recent exposure, feathers can better monitor cumulative exposure, at least during the seasonal life of the feather (before molting), and have the additional advantage of noninvasive biologic sampling. In addition, metal excretion through feathers can be significant, and its utility in estimating exposure in wild caught individuals can be complex. It includes such factors as variability of exposure, variability of feather growth/molt, and the vagile nature of many bird species.

Both kidney and feathers accumulate Pb, and although our study showed an 8-fold difference between concentrations in feather and kidney, others have shown that values were almost equal in dosed zebra finches (Dauwe et al. 2002). Given the different timescales of other studies, it is difficult to assess the significance of this difference, but part of it could be due to our measurement of metals in dried tissues samples. Although the dose–response effect in feathers was also evident for soils amended with tungstate, the magnitude of the effect was decreased by half, further evidence that tungstate somehow interferes with Pb absorption. However, this should be qualified with the evidence from the NaCl treatment (Fig. 2a), which shows that NaCl caused decreased blood Pb concentrations and therefore could also have been a factor in the decreased feather Pb concentrations. Although tungsten nylon (which degrades to tungstate) has not been fielded by the military in small arms, this interesting observation shows that anions in the soil could affect bioaccessibility and hence the uptake of toxic metals such as Pb. This antagonistic effect should be considered when designing absorption, distribution, metabolism and excretion, or bioavailability studies of metals.

Interestingly, doses of highly soluble PbAc, similar in concentration to Pb doses in soil, were not absorbed in a dose–response manner (Fig. 2a). In fact, doses of 2500 and 5000 μg Pb (as acetate) were indistinguishable when measured in blood (Fig. 2a), but they did show a significant dose–response effect in tissues (Table 2). Tissues accumulate Pb over time, whereas blood levels reflect recently absorbed Pb (by way of Pb in erythrocytes), which is then distribution to tissues. PbAc (or other soluble salt) has often been used to establish benchmark Pb toxicity and has

also been used as a reference level for studies on bioavailability (Bannon et al. 2009; Casteel et al. 2006). Data from the present study suggest that in birds, similar to swine (Bannon et al. 2009), the relative bioavailability of Pb from SAR soil is similar to that of PbAc, at least for the low-dose treatment (compare PbAc with soil in Table 1).

In conclusion, Pb was effectively absorbed from SAR soil in a dose-dependent manner in pigeons according to blood, tissues, and feathers measurements. Increased EP values indicated incipient Pb poisoning, although other hematopoietic components were undisturbed. Although soil amended with Cu or tungstate showed decreased tissue Pb levels, these may have been confounded by their respective anions or cations. Newly grown feathers reflected the dose–response patterns of blood and tissue. At least for the short-term period of this study, pigeons were able to tolerate high internal doses of Pb.

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